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Keynote Address: Potential of *in Vitro* Tests in Asbestos-Related Problems

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Clarification of particle-cellular interactions, in what Policard called the "conflict of living matter with the mineral world," (*1*) has had slow development. Even the special potential of crystalline silica (quartz) was not fully understood until Collis' classic description of its unique biological properties in his Milroy lecture of 1915 (*2*). It was known that some individuals exposed to dust suffered severe lung damage (the knife grinders of Sheffield, for example). But it was equally known that others exposed to "dust" had no such consequence. Farm laborers in England, who worked in clouds of dusts, had the advantage of long life spans. Evidently, there were dusts and dusts, reacting differently with tissues. This was confirmed when Collis observed that workers exposed to quartz died much more frequently of tuberculosis than those with other kinds of dust exposure. Even against this background, elucidation of the mechanisms of silica's biological properties is still incomplete; while we have advanced our knowledge of these particles' intracellular activity (*3*), much study is now directed to membrane effects (*4*) and the question of immunological mechanisms (*5*).

Problems associated with the activity of fibrous silicate minerals have remained incompletely explored, with equal delay. The first patient of whom we have record in whom asbestos was appreciated to be a health problem was an asbestos factory worker seen by H. Montague Murray in 1898 at the Charing Cross Hospital in London. The man died the next year of respiratory insufficiency. An autopsy showed diffuse pulmonary fibrosis. The death was commented on in the records of a Departmental Committee of the British Parliament in 1906 (*6*). There were additional scattered references to the possibility of asbestos-associated respiratory disease in the following years (*7*), but the first documented case report did not appear in the medical literature until 1924 (*8, 9*). The spectrum of asbestos-associated disease was extended in the next five decades to include not only asbestosis (parenchymal and pleural fibrosis) but a number of neoplasms, with increased incidence of lung cancer, pleural and peritoneal mesothelioma, cancer of the esophagus, stomach, colon-rectum, pharynx and buccal cavity, larynx and kidney (*10*). Unfortunately, there has been considerable disparity between the clinical and pathological observations that have been made, and our understanding of the mechanisms and cellular processes related to these observations. It may therefore be useful to outline some of the critical questions that characterize this imbalance.

A basic question relates to the problem of interstitial fibrosis. While is has been known for more than 80 years that this can develop following asbestos exposure, it is still not understood why. For example, not all individuals who inhale asbestos will develop fibrosis, and among those who do, it often develops in different degree, despite equal exposure and equal tissue residence. In some individuals, there will be minimal fibrotic reaction; in others, the changes are much more extensive. Some show most damage in the parenchyma; others have little interstitial change, but much pleural fibrosis. A number of explanations have been offered, acting singly or in combination, ranging from the nature of the fibers [mineral type, length, diameter, surface, crystal structure, etc. (*11*)] to individual susceptibility. While a variety of mineral dusts will produce parenchymal fibrosis, it appears that only asbestos and a few other materials, viz., zeolites (*12*), have the capacity to produce pleural change in the form of localized or diffuse pleural fibrosis and/or calcification. It is not that other dusts do not reach the pleura. Particles of iron oxide instilled into the pleural cavity were shown in experiments more than 50 years ago to enter the parietal pleura, and black reticulation of the pleura in coal miners is commonplace. Yet little is so far known concerning cellular reactions of the mesothelial surfaces of the chest (or of

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the peritoneal cavity!) to help us understand why both fibrotic and neoplastic disease can occur following the inhalation of some fibrous microparticles. Once more, one is struck by the range from minimal to extensive disease and, once again, the question of individual susceptibility must be addressed. Appropriate *in vitro* test systems should prove particularly useful in allowing better understanding of mesothelial cell response to particulate matter.

In developing such systems, it would be particularly advantageous if the problem of long clinical latency were to be faced. Clinically, this is well known and well accepted. Radiological evidence of fibrosis/calcification usually does not begin to appear for 20 years or more following onset of exposure, although some cases are seen earlier (*13*). Even though it is appreciated that histological and even gross anatomical changes may be present before X-ray abnormalities, there is still a period of many years before such alteration is noted. It is also appreciated that there may be long continued tissue residence of inhaled fibers, once inhaled. Nevertheless, we do not understand the cellular-particle interactions that explain the decades-long continuum of histological development. This is equally true with neoplastic change in the mesothelial surfaces, where mesothelioma generally does not become clinically evident until 20 to 40 years or more after initial exposure (*14*). Better understanding of what is happening at the cellular level is needed to understand the years-long progression and development of disease, both with continuing exposure or following limited, short-term exposure with subsequent passage of time and later occurrence of disease (*15*).

The problem of neoplastic change following asbestos exposure has overtaken that of fibrotic reaction, and asbestos-associated neoplasms have taken pride of place in public health concerns related to asbestos exposure (*16*). Among occupational groups, lung cancers are the most important asbestos-associated neoplasms, with mesothelioma following close by. With lower levels of exposure, as seen in environmental circumstances, mesothelioma has gained prominence, although we have few data concerning increased incidence of bronchogenic carcinoma with such exposure. Lung cancer developing following exposure to asbestos was first reported by Lynch and Smith in 1935 in the United States (*17*), and, in the same year, the first asbestos-related lung cancers were also reported in Great Britain (*18*). In 1960, Wagner, Sleggs and Marchand emphasized the relationship between pleural mesothelioma and asbestos exposure (*19*). More recently, various other neoplasms have been found to occur in increased incidence among asbestos-exposed workers (*10*). Nevertheless, although asbestos reaches virtually every tissue in the body, only some organs show a neoplastic response. Other organs, similarly exposed, demonstrate no malignant change. For example, A. M. Langer in our laboratory has demonstrated myriads of asbestos fibers in livers of asbestos workers; we do not find an increased incidence of primary hepatocellular carcinoma in cohort studies of asbestos workers (*10, 20*). There does not seem to be a statistically significant increased incidence of leukemia or lymphoma, and cancer of the bladder occurs at about expected levels, despite cancer of the kidney being increased (*10*). Some tissues do and some tissues do not react to asbestos with neoplastic change; study of the cellular reactions to the fiber might therefore include analysis not only of cellular neoplastic change in general, but organ differences as well.

Dose-disease response is ever with us in studying environmental disease. It is surely true with asbestos. It is now appreciated that significant pulmonary fibrosis is unlikely unless relatively large amounts of asbestos have been inhaled. Lesser intensities and duration (dose), however, can result in diffuse interstitial fibrosis of lesser degree. With even smaller amounts of asbestos, as often obtained in environmental circumstances (viz., family contact or neighborhood disease), pleural change alone may be seen, with very little noted in the lung parenchyma. Differences associated with intensity and duration of exposure also result in differences in relative frequency of the various asbestos-associated neoplasms. It is generally agreed that pleural mesothelioma can occur with comparatively little asbestos, surely at the levels which occurred in households of asbestos workers in the past. We do not know if there is a lower limit of asbestos exposure that would not be associated with an increased occurrence of pleural mesothelioma. Our own experience indicates that there is dose-disease response gradient for pleural mesothelioma. It is appreciated that epidemiological confirmation of such a gradient at very low levels of exposure (e.g., ambient air contamination) will be difficult. In any case, *in vitro* studies would be particularly valuable if information could be provided concerning disease potential at low levels of exposure. The public health importance of such studies is emphasized by observations of the frequency of asbestos-associated disease among family contacts of asbestos workers (*21*). In one group, approximately one-third of wives and children developed parenchymal/pleural changes, and initial observations suggested that approximately 1% of deaths among family contacts 20 years or more following onset of the workers' employment were due to pleural mesothelioma. Parenthetically, clinical observation suggests that the dif-

ferent mesothelial surfaces respond differently in terms of dose. Pleural mesothelioma is often a problem of "low" exposure, in environmental circumstances, whereas peritoneal mesothelioma is far more likely to occur with the heavier exposures of the work place. Is the cellular response of the pleural mesothelial cells different than those of the peritoneum? Are differences in fiber mass, number, size responsible, or are there important physiological differences (pleural drift, in the lung)?

Clinical and epidemiological observations have provided another conundrum that can be profitably explored in *in vitro* studies—the multiple factor interactions between asbestos exposure and cigarette smoking. Both, individually, increase the risk of lung cancer. Nonsmoking asbestors workers have approximately five times the risk of dying of lung cancer compared to nonsmokers in general, whereas those who smoke also have augmentation of their risk, compared to smokers who are not exposed to asbestos (*22, 23*). Thus, it is not a question as to whether asbestos alone can "cause" lung cancer; it can. But from a public health point of view this important potential is not strikingly demonstrated unless there is concomitant cigarette smoking. For nonsmokers, baseline lung cancer risk is generally low, and even multiplying it five times by the inhalation of asbestos still does not result in a very large number of neoplasms. On the other hand, multiplying the already very high risk of cigarette smokers results in the extraordinary lung cancer incidence that we find among asbestos-exposed workers; in some cohorts, 20% of all deaths are due to this neoplasm (*20, 23*). It appears that similar interaction underlies the increased incidence of cancer of the esophagus, pharynx and buccal cavity and larynx (*23*). On the other hand, there is no such effect for pleural or peritoneal mesothelioma, cancer of the stomach or colon-rectum, or kidney cancer. Smoking and nonsmoking asbestos workers have equal risk. Two problems therefore need investigation: the nature of the cellular response to the carcinogens, singly and together, culminating in the neoplastic change and the nature of differences in cells of different organs in their varying response to the combination of cigarette smoke and asbestos.

Recent observations have added a third problem. It has been found that asbestos workers who stop smoking can, within 5 to 10 years, reduce their risk of dying of lung cancer to approximately one-half or one-third that of their colleagues who continue to smoke (*24*). Thus, while the lung cancers that occur among asbestos workers who smoke are not reversible once they occur, the risk of developing such neoplasms can be sharply reduced by cessation of cigarette smoking. The cellular developments which underlie such a reversal of risk are unknown, and their clarification would constitute a major advance.

An overriding question in much of environmentally induced disease is the nature of the interaction between individual biological constitution (? genetic) and exogenous agents. This is clearly the case with asbestos. If we say that 1 in 15 insulation workers dies of mesothelioma, we are concomitantly saying that 14 do not. One in five dies of lung cancer, four escape this fate. Pulmonary asbestosis of sufficient severity to cause fatal pulmonary insufficiency has only been seen in approximately 7% of asbestos insulation workers; some, even after 40 years or more of work in their trade, have normal or virtually normal chest X-rays (*25*). The nature and background for such variations in individual response remain obscure. In our laboratory, with J. G. Bekesi, we have been investigating occurrence of immunomodification associated with asbestos exposure (*26*). It has been found, as expected, that virtually all patients with mesothelioma are markedly immunosuppressed. But a few are not, and the curious observation has been made that those who have survived more than the usual year or less have been those with normal or virtually normal immunological status. Of course, there is no necessary cause and effect relationship between these two observations. Nevertheless, the theoretical connotations seem important, and we are currently investigating the immune status of a very large number of asbestos insulation and asbestos factory workers, virtually all of whom are 30 years or more from onset of their work. Initial investigation of a group of more than 200 men showed lesser or greater degrees of immunosuppression in approximately one-third. We are particularly interested in the prognostic significance of variations in the immune state. We consider that the entire area of variation in immunological status associated with exposure to asbestos fibers requires close study at the cellular and serological levels; it is likely that such studies hold great promise in a very rapidly developing field.

Our inadequacies concerning basic questions related to asbestos-associated disease are particularly disadvantageous at this time. As a result of exposure in the past, much asbestos disease is anticipated in the next decades. Nicholson and his colleagues have calculated that among workers exposed from 1940-1979 in a number of important trades and industries (asbestos mining and milling and factory workers, construction, shipbuilding and ship repair, chemical plants, refineries, utilities and power production, transportation, among others) there will be approximately 9000 excess deaths of asbestos-associated cancers annually until the turn of the century, with a lessening incidence thereafter

(*27*). These projections do not include deaths of asbestosis nor deaths of asbestos-associated disease among family contacts of asbestos workers or among those exposed in environmental circumstances. Nor do they include individuals who suffer asbestosis, but do not die of this disease. Our ability to cope with what Doll and Peto have called "... this public health disaster" (*28*) is currently limited. Better understanding of the basic mechanisms may allow us to significantly improve management and treatment of those who have been exposed to asbestos in the past and who now face an uncertain future.

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